(2)

AD-A223 539

DOCUMENTATION PAGE

Form Approved OMB No 0704-0188

1a SECURITY CLASSIFICATION AUTHORITY: NA	1b RESTRICTIVE MARKINGS: NA
2b DECLASSIFICATION / DOWNGRADING SCHEDULE: NA	3 DISTRIBUTION / AVAILABILITY OF REPORT: Distribution unlimited
4 PERFORMING ORGANIZATION REPORT NUMBER(S): Boston University	5 MONITORING ORGANIZATION REPORT NUMBER(S): NA
6a NAME OF PERFORMING ORGANIZATION: Boston University; 6b OFFICE SYMBOL (If applicable): NA	7a NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c ADDRESS (City, State, and ZIP Code): Chemistry Department, Boston University, 590 Commonwealth Avenue, Boston, MA 02215	7b ADDRESS (City, State, and ZIP Code): 800 N. Quincy St., Arlington, VA 22217-5000
8a NAME OF FUNDING / SPONSORING ORGANIZATION: Office of Naval Research; 8b OFFICE SYMBOL (If applicable): ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-89-J-1756
8c ADDRESS (City, State, and ZIP Code): 800 N. Quincy St., Arlington, VA 22217-5000	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO: 61153N; PROJECT NO: RR04106; TASK NO: 441p014; WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification)

(U) Characterization of Marine Bioadhesive Proteins

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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Progress	FROM 7/1/89 TO 6/30/90	1990 July 1	5

16 SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Adhesive, Proteins, Mussel, Amino Acid Sequence, Gene Sequence, cDNA, Marine Bioadhesive Proteins, Biochemistry, Wet Surfaces

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The objective of this research is to elucidate the amino acid sequences, via gene sequencing, of the adhesive proteins from several species of mussel, with the aim of understanding how these organisms attached themselves to wet surfaces. During the past year we have completed sequencing cDNA clones that code for the entire amino sequence of the adhesive protein of *Mytilus edulis* and for the C-terminal approximately 75% of the adhesive protein of *Geukensia demissa*. In addition, we have obtained sequence information on a new *Geukensia* protein that has a 47-amino acid repeat sequence. The patterns of repeat sequences in both adhesive proteins suggests to us that the conformations of the repeat peptide domains is important for function, but that the overall arrangement of the domains is less so.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(U)
22a NAME OF RESPONSIBLE INDIVIDUAL: M. Marron	22b TELEPHONE (Include Area Code): (202) 696-4760; 22c OFFICE SYMBOL: ONR

DD Form 1473, JUN 86 — Previous editions are obsolete — S/N 0102-LF-014-6603 — SECURITY CLASSIFICATION OF THIS PAGE

ANNUAL PROGRESS REPORT

GRANT#: N00014-89-J-1756 R&T CODE: 441p014

PRINCIPAL INVESTIGATOR: Richard A. Laursen

INSTITUTE: Boston University

GRANT TITLE: Characterization of Marine Bioadhesive Proteins

PERIOD OF PERFORMANCE: 1 July 1989 - 30 June 1990

OBJECTIVE: To complete sequence studies on cDNA clones encoding the amino acid sequences of the adhesive proteins of the mussels, *Mytilus edulis* and *Geukensia demissa*.

ACCOMPLISHMENTS (last 12 months): During the past year we have completed determination of the amino acid sequence of the adhesive protein of the mussel, *Mytilus edulis*. The sequence consists of 911 amino acids including a 24-residue signal sequence. The amino acid sequence was obtained by sequencing a 2940-bp cDNA clone, which gave the entire coding sequence, except for the start codon. The remainder (the 5' end) was obtained from a second clone which was amplified using the polymer chain reaction. A key to obtaining large clones was transforming the cDNA library into the rec^- host, *E. coli* CES201. Comparison of our sequence with a genomic sequence at obtained Genex Corp. reveals differences. Both proteins contain about 84 decapeptide and hexapeptide repeats and have identical sequences at the N-terminal and C-terminal ends, but there is a variation in the pattern of repeat units and in the sequences of some of the decapeptide repeats in the middle part of the protein.

We have also completed sequencing two cDNA clones from *Geukensia demissa*, which contain 1900 and 1550 basepairs, the larger of which codes for 557 amino acids. As reported earlier, the *Geukensia* sequence is more complex, consisting of repeats of an octapeptide, and one of two pentapeptides or a tripeptide. As with *Mytilus*, the sequences are identical at the 3' end (including the non-coding region), but the pattern of repeats differs toward the middle of the protein. We have not yet obtained the sequence of the 5' end.

Geukensia clones were selected by immunoscreening in an expression vector. One of the clones isolated by this procedure had a sequence completely different from from any adhesive protein of known sequence. It had a repeat sequence of 47 residues with cysteine residues near each end of the repeat unit, suggesting multi-loop structure. Northern blot studies in which mRNA was hybridized with synthetic probes from the known adhesive protein sequence and from the new protein sequence indicated that the 47-mer repeat protein is not a part of the adhesive protein, i.e., there was no cross-hybridization. Most likely this new protein copurified with the adhesive protein used in raising antibodies. The repeat sequence suggests it is a structural protein, but a search of the protein sequence database revealed no obvious similarities with other proteins.

SIGNIFICANCE: The finding of multiple sequences for both the *Mytilus* and *Geukensia* adhesive proteins could have at least three causes: recombination during cloning, multiple genes within an organism, and population diversity. We now favor the latter explanation, though further study is required to demonstrate it. If this is the case, it suggests that that the precise arrangement of peptide repeats in the protein is not very important for function. On the other hand, certain features in the repeat sequences are

essentially invariant, suggesting that the repeat domains possess a specific conformation that is required for function.

WORK PLAN (next 12 months): Inasmuch as funds for this project are now nearly exhausted, we will concentrate on writing up the results of these studies.

INVENTIONS (last 12 months): None

PUBLICATIONS AND REPORTS:

Lectures:

"Characterization of Mussel Adhesive Proteins"

ONR Contractors Meeting, Belmont, MD, 11/11/89
Materials Research Meeting, Boston, MA, 11/30/89
Hunan Biological Research Institute, Changsha, Hunan, P.R. China, 5/3/90
Department of Biology, Peking University, Beijing, P.R. China, 5/10/90
Cancer Research Institute, Bombay, India, 5/15/90

Articles:

Characterization and Structure of Mussel Adhesive Proteins, R.A. Laursen, J.J. Ou, X.T. Shen and M.J. Connors, in *Materials Synthsis Utilizing Biological Processes,* Materials Research Society, Pittsburgh (in press).

ANNUAL REPORT QUESTIONNAIRE

Principal Investigator:	Richard A. Laursen
Institute:	Department of Chemistry Boston University 590 Commonwealth Ave. Boston, MA 02215
Grant title:	Characterization of Mussel Adhesive Proteins
Period of performance:	1 July 1989 - 30 June 1990
Number of pulications last year:	One (in press)
Number of patents/inventions:	None
Total number of students/trainees:	Two
How many are female?	Two
How many are minority students?	Two (Chinese)
How many are not US citizens?	Two (PRC and ROC)
Awards/Honors to PI	Elected Fellow of the American Association for the Advancement of Science.
Equipment purchased:	None
Email address:	Laursen@bu-chem.bu.edu (Internet)

HIGHLIGHTS

CHARACTERIZATION OF MARINE BIOADHESIVE PROTEINS

Richard A. Laursen
Boston University, 1990

Objectives

- Determine the amino acid sequence of the mussel adhesive proteins
- Understand how protein amino acid sequence affects function
- Use knowledge of adhesive protein structure to design wet-surface adhesives

Accomplishments

- Completed sequence (via cDNA sequencing) of *Mytilus edulis* adhesive protein
- Obtained C-terminal 75% of *Geukensia demissa* adhesive protein sequence
- Obtained partial sequence of new repetitive structural(?) protein from *G. demissa*

Adhesive protein partial sequences showing typical patterns

M. edulis	*G. demissa*
AKPSYPSTYK	GKPSSYDPGYK
AKSSYPPTYK	GQQKQTGYDTGYK
AKP TYK	GQQKQTAYDPGYK
AKPTYPSTYK	GGVKKTGYSADYK
AKPSYPPTYK	GKPSSNVPGYK

Significance

- Structure of repeat peptide domain is important for function
- Pattern of repeat domains in intact protein is less important